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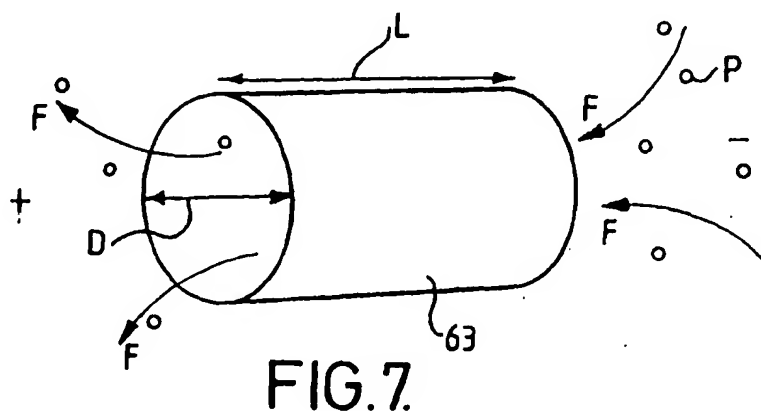
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(54) **Particle sizing apparatus**

(57) Apparatus for determining the size of a particle or cell within a fluid, comprising a sample chamber for the fluid, a flow restrictor comprising an orifice, a pair of electrodes disposed on opposite sides of the orifice and means for measuring a signal representative of the impedance variation between the electrodes thereby to determine the size of a particle within the fluid passing through the orifice, and further comprising means for detecting blockage of the orifice, whether partial or com-

plete. Apparatus for determining the size of particles or cells within a fluid, comprising a sample chamber for the fluid, a flow restrictor comprising an orifice, a pair of electrodes disposed on opposite sides of the orifice and means for measuring a signal representing the impedance variation between the electrode thereby to determine the size of a cell within the fluid passing through the orifice, and further comprising means for deblocking the orifice by causing movement of a particle at least momentarily held within the orifice.



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## Description

The invention relates to particle or cell sizing and counting apparatus and to methods of operation thereof. In particular, the invention relates to apparatus which uses a technique of measuring the impedance at an orifice to determine the volume of a particle passing through the orifice.

It is known from EP 0162607 to determine the size of a particle from the variation in impedance between a pair of electrodes in an electrolyte due to particle flow through an orifice in a flow restrictor disposed between the electrodes. An inherent problem of this type of system however, is that partial or complete blockage of the orifice can occur during measurements which requires that the flow restrictor comprising the orifice must be removed in order to be cleaned to allow further measurements. Additionally, if only partial blockage occurs the observed distribution of particle sizes in a sample will be affected due to prevention of flow by the blockage of larger particles through the orifice. This problem is particularly prevalent if one wishes to use a small orifice diameter of say five times the average particle size to enable good accuracy of particle sizing results.

The invention seeks to avoid or at least mitigate problems of the prior art including providing apparatus which detects blockage and or deblocks the orifice especially in the event of partial or complete blockage.

According to first aspect of the invention there is provided apparatus for determining the size of a particle or cell within a fluid, comprising a sample chamber for the fluid, a flow restrictor comprising an orifice, a pair of electrodes disposed on opposite sides of the orifice and means for measuring a signal representative of the impedance variation between the electrodes thereby to determine the size of a particle within the fluid passing through the orifice, and further comprising means for detecting blockage of the orifice, whether partial or complete. Beneficially the detection of a partial or complete blockage alerts the user to data corruption.

Preferably, the detecting means comprises means for monitoring the signal, which means detects occurrence of a predetermined variation of the signal indicative of blockage of the orifice. For example, particle passage through the orifice causes a signal pulse which is measured by the measuring means and wherein the monitoring means determines a width of the signal pulse and compares this width with a predetermined value thereby to detect partial or complete blockage of the orifice. The predetermined pulse width value can be determined from an average of previously measured pulse widths.

In another form, the monitoring means monitors the mean base line value of the signal to determine if a significant drift in mean base line value occurs which is indicative of partial or complete blockage of the orifice.

Alternatively or as well, the monitoring means compares the height of an individual signal pulse with a

known value corresponding to a particle size in the order of or greater than a predetermined size such as the diameter of the orifice. Also, the monitoring means can comprise a saturation pulse or square wave detector.

The square wave detector can compare the time for a pulse signal to pass through a first and second predetermined value and then return back through the predetermined first and second values. The square wave detector can be arranged to detect a characteristic recovery curve indicative of saturation of the measuring means.

In another form, the monitoring means measures the rate of occurrence of signal pulses and compares this rate with a predetermined rate. Also, the monitoring means can compare the number of detected signal pulses in a given time interval with a predetermined value, such as an average of previous measurements.

Beneficially, the monitoring means can analyse the background noise of the signal for predetermined variation, such as amplitude variation within a frequency range.

Further, the detecting means can comprise an orifice current detector for determining electrical current flow between the electrodes thereby enabling the monitoring means to compare orifice current with predetermined values. The detecting means can compare the orifice current value before or after measuring a signal or the mean of the two with an initial value measured before the signal measurement. The detecting means can determine if the difference is greater than a 10% increase or a 5% decrease, or if the difference is greater than 20% say.

Preferably, the measuring means comprises a 14 bit detector, or an analogue to digital converter having 14 bit resolution. Also, the apparatus preferably comprises means for applying a calibration signal to one of the electrodes and the monitoring means the signal across both electrodes, the monitoring means being adapted to compare the detected calibration signal with a pre-determined signal. The calibration signal can comprise a series of pulses. The calibration signal can be repeated a predetermined number of times to attempt to obtain an acceptable result with the predetermined signal, before providing an alarm to the user. Also, means for deblocking the orifice by removing a particle at least momentarily held within the orifice can be provided.

According to another aspect of the invention there is provided apparatus for determining the size of a particle or cell within a fluid, comprising a sample chamber for the fluid, a flow restrictor comprising an orifice, a pair of electrodes disposed on opposite sides of the orifice and means for measuring a signal representative of the impedance variation between the electrodes thereby to determine the size of a particle within the fluid passing through the orifice, and further comprising means for deblocking the orifice by causing movement of a particle held at least momentarily within the orifice. Beneficially

the deblocking means can effect in situ removal of the blockage, therefore, the apparatus allows continuous sampling without requiring the user to dismantle the sample chamber or remove the flow restrictor for cleaning when a blockage occurs.

Preferably, the deblocking means operably creates ultrasonic vibrations in the fluid in the region of the orifice. A series of electrical pulses can be applied to one of the pair of electrodes. The pulse frequency is preferably greater than 5 kHz, preferably in the order of 10 kHz but ultrasonic frequencies greater than 15 kHz, and possibly up to about 20 kHz. Beneficially, the flow restrictor can comprise material that exhibit the piezo-electric effect, thus to enhance the deblocking effect in the presence of electrical pulses.

Also, the deblocking means can comprise fluid propulsion means for effecting fluid flow at the flow restrictor. Preferably, the fluid propulsion means directs fluid substantially in the reverse direction to fluid flow during signal measurements.

Beneficially, means within the chamber can be used for directing fluid from the fluid propulsion means towards the flow restrictor. For example, a tapered region of the chamber can be used.

Another aspect of the invention provides a sample chamber housing for particle sizing apparatus comprising a pair of recesses each adapted to receive an electrode, an electrode in each recess, and a two-part sealant and adhesive for the electrodes. The adhesive can comprise a structural acrylic adhesive. The sealant can comprise a silicone based sealant such as RTV silicon rubber. The recess is preferably defined in a body of a polymer such as acrylic.

A further aspect of the invention provides a flow restrictor for particle sizing apparatus, comprising an orifice allowing flow of particles there through which orifice is tapered. The taper is preferably in the order of 5 to 30 % of the mean orifice diameter.

A further aspect of the invention provides a flow restrictor wherein the material defining the orifice is flexible.

According to a yet further aspect of the invention there is provided a sample chamber housing for particle sizing apparatus comprising a first chamber and a second chamber for holding liquids, a liquid communication passage between the first and second chambers adapted to receive a flow restrictor, a sample inlet into the first chamber, and wherein the housing also defines an outlet from the first chamber to enable automatic removal of samples therefrom. Also, the invention provides a particle sizing apparatus comprising a sample chamber housing of this type. The housing and apparatus can comprise various features as will become apparent from the following description which enable automated control of the input of sample into the first chamber, dilution of the sample, washing of the first and second chambers, and automatic removal of liquids in the first or second chamber.

The next further aspect of the invention provides a particle sizing apparatus comprising a first and second chamber separated by a flow restrictor comprising an orifice, means for measuring a signal pulse from the variation in electrical impedance at the flow restrictor due to the presence of a particle in the orifice, and means for detecting a partial or complete blockage of the orifice which detecting means comprises at least two, and optionally enabling selection of any two from at least three, of the following:-

- means for monitoring the rate of signal pulse detection,
- means for comparing the width of a signal pulse or an average of signal pulses, with a predetermined value such as a static limit or a cumulative average of earlier signals,
- means for measuring a change in electrical current across the flow restrictor,
- means for comparing a detected particle size with a predetermined value such as the size of the orifice,
- means for determining the presence of a saturation signal, or
- means for determining anomalies in the background noise.

A deblocking mechanism according to an aspect of the invention, can be effected through a single effect or combination of the following effects, namely:-

- the application of a high frequency electrical signal, for example in the order of 10 kHz, across an orifice of a flow restrictor to cause a piezo-electric effect in a flow restrictor comprising piezo-electric material, such as a ruby crystal, simple application of such a signal, or higher signal, for example in the order of 15 to 20 kHz, to cause an acoustic effect within the fluid proximal the orifice in a flow restrictor, removal and re-application of a dc voltage between a pair of electrodes on either side of a flow restrictor, such as about 30 volt dc voltage, or reversing fluid flow through the orifice for example using a jet of fluid directed at the orifice.

Embodiments of the invention will now be described by way of example only, with reference to the accompanying drawings in which;

Figure 1 is a schematic perspective view of a sample wand and a sample chamber housing forming part of the apparatus according to the invention;

Figure 2 is a schematic front elevation view of the sample wand alignment guide as shown in figure 1;

Figure 3 is a schematic block diagram of the fluid control apparatus according to the invention;

Figure 4 is a sectional view through a sample chamber according to the invention;

Figure 5 is an end view from inside the apparatus of the sample chamber shown in figure 4;

Figure 6 is a partial sectional view of the sample chamber shown in figures 4 and 5;

Figure 7 is a schematic perspective view of an orifice in a flow restrictor;

Figure 8 is a schematic perspective view of a second flow restrictor according to the invention;

Figures 9a, b and c are is a schematic side views of the restrictor of Figure 8 in three different modes of use.

Figure 10 is a schematic block diagram of part of the electronic control system for the apparatus according to the invention;

Figure 11 is a circuit diagram for part of the amplification circuit for the apparatus according to the invention; and

Figure 12 is a schematic view of a signal pulse detected and analysed by the apparatus according to the invention.

Referring to figure 1 there is shown part of a particle sizing apparatus 10 according to the invention comprising a main housing 12 from which protrudes a sample chamber housing 14 having an inlet 16 through which a sample passes into first chamber 52 shown in figure 4. Additionally, a wand guide 18 protrudes from housing 12. Wand guide 18 comprises a vertical groove or channel 20 which enables alignment of nozzle 24 of hand-held wand 26 with inlet 16. Hand-held wand 26 further comprises a replaceable tip 28, a button 30, fluid inlet pipe 32 and electrical connection 34. As shown in figure 2 wand guide 18 comprises a pair of detectors 22 such as optical detectors to determine if wand nozzle 24 is in place within groove 20.

Referring to figure 3 there is shown a schematic drawing of the fluid control system 36 used in apparatus 10. In fluid control system 36 a diluent reservoir 38, which can house say three litres of electrolyte for example, is connected along one path to a three-way valve 40 which is connected at one outlet to a syringe drive 42 which can comprise a 12 volt stepper motor for example, and at another outlet is connectable to wand 26 by fluid inlet pipe 32.

Reservoir 38 is also connected via a T-connector

44 to a diluent sensor 41, which can be an optical device for determining if diluent is present. A pump 46 such as a 12 volt DC peristaltic pump, drives diluent to sample chamber housing 14 via a Y-connector 48.

One outlet of Y-connector 48 is connected to tube 50 having a valve 51 such as an electrically operated pinch valve, which controls flow of diluent through tube 50 to a first chamber 52 forming part of sample chamber housing 14. The other outlet from Y-connector 48 passes through tube 54 which has a valve 56, again such as an electrically driven pinch valve, which controls flow of fluid through tube 54 into a second chamber 58 of the sample chamber housing 14. First and second chambers 52 and 58 respectively, are separated by a flow restrictor 60 having an orifice 63 (see figure 7, 8 and 9). Sample chamber 14 is shown in greater detail in figures 4 to 6.

System 36 further comprises a suction system comprising a pair of vacuum pumps 62. Each pump is connected to a vacuum reservoir 64 via a valve 66, such as an electrically driven pinch valve. Purge tubes 68 pass almost to the bottom of reservoir 64 thereby to enable expulsion of any liquid in reservoir 64 via at least one of valves 66, pump 62 and outlet 70 thus for disposal.

Reservoir 64 is further connected via tube 72 to the second chamber 58 of housing 14. Tube 72 further comprises a valve 74 such as a pinch valve and a pressure transducer 76 such as a Honeywell Controls type 141PC05G device. Reservoir 64 is further connected to the first sample chamber 52 via tube 78 having a valve 80.

Referring to figures 4 to 6, sample chamber housing 14 is shown which preferably comprises an outer shield 15 against electromagnetic radiation such as a metallic casing. Met 16 leads to first chamber 52 which is separated from second chamber 58 by flow restrictor 60. The chambers can be made in a body of inert material such as an acrylic or other plastic. The flow restrictor can comprise a crystalline material such as ruby, sapphire, or alumina or a polymer having a orifice 63. In one preferred form the restrictor comprises a piezo-electric material. As shown in figure 7 orifice 63 has length L and diameter D, for example a length of 80 microns and diameter of 30 microns is preferred for certain sizes or volumes of particle P which during measurement flow through orifice 63 along direction F. However, other sizes of orifice are possible. Diluent tube 50 enables diluent to pass into first chamber 52 whilst tube 78 enables evacuation of the first chamber.

Similarly tube 54 allows diluent to pass into second chamber 58 and tube 72 enables the evacuation thereof. In practice the tubes can comprise different types of passageways, channels and connectors as appropriate. Preferably tubes 50 and 54 are arranged to direct liquid at the flow restrictor 60. Inlets to sample chamber housing 14 are shown in figure 5.

In figure 6, it can be seen that a recess 61 can be provided for holding flow restrictor 60 thereby to sepa-

rate chamber 62 and 58. O-rings can also be used to seal the edges of flow restrictor 60. Additionally, electrodes 82 and 84 are shown effectively on opposite sides of flow restrictor 60, i.e. one in each of chambers 52 and 58. The electrodes can be made of platinum for example and are connectable to an electronics control system via connector 86.

It has been found particularly useful to attach electrodes 84 and 82 to housing 14 using a two-part sealant and adhesive. This is in order to prevent leakage of fluid from chambers 51 and 58 and maintain the electrode in a correct position in spite of attaching and detaching chamber housing 14 from main housing 12. Preferably, the electrodes are mounted in a recess in housing 14 which can be made of acrylic or other polymer material. The upper part of the recess, that is adjacent the fluid chambers, is sealed with a water resistant and vacuum seal material such as a silicon based sealant, for example RTV silicone rubber. A second stage of adhesive is placed below the sealant to attach the electrode to the walls of the recess. A suitable adhesive is a structural acrylic adhesive. In a preferred form, chamber housing 14 is made from acrylic, the electrodes have an outer platinum coating or are made of platinum and the two-stage sealant and fixing comprises RTV silicone rubber and structural acrylic adhesive.

Preferably, chamber housing 14 is easily detachable from main housing 12. Accordingly, electrical connector 86 is preferably a simple bayonet-type connector comprising male components adapted to fit into a female socket on housing 12 for example. Additionally, as shown in figures 4 and 5, tubes 72, 54 and 78 allow for easy mounting on housing 12 and can for example be the female part of a male/female connection. Accordingly, chamber housing 14 can be attached and removed from main housing 12 in a simple push/pull operation.

Referring to figures 8 and 9 there is shown a second embodiment of a flow restrictor 60 according to the invention wherein orifice 63 is tapered. For example, the aperture on one side of restrictor 60 could be 32 to 38 microns in diameter whilst the orifice on the other side could be 30 microns. Accordingly, a taper of say 2 to 8 microns in the diameter of the orifice is affected across its length of say 80 microns. Preferably, the narrower orifice faces the second chamber 58 such that in use, as shown in figure a, where the restrictor 60 is made of resilient material, it flexes creating an orifice 63 having approximately parallel sides thereby enabling laminar flow through the orifice. The rest position is shown in figure b and a blow-back or clearing operation position is shown in figure c. The clearing operation is described in more detail later. Preferably, the flow restrictor is made of a polymer such as a non-fluoride polycarbonate such as PET or polystyrene. The orifice can be laser etched into the flow restrictor.

Preferably, to assist in cleaning a blocked orifice 63, second chamber 58 comprises a tapered region 59 which narrows towards flow restrictor 60 (see figure 4).

Additionally, preferably tube inlet 54 to chamber 58 is axially aligned with orifice 63 so that fluid passing into chamber 58 is directed to effect turbulence at flow restrictor 60 and possibly to cause reverse flow of diluent through to first chamber 52. The tapering of chamber 58 assists in this reverse flow and additionally causes circulation of diluent in chamber 58 assisting in the cleaning thereof. Further, a flexible restrictor 60 provides the enhanced clearance configuration shown in figure c.

Referring to figure 10 there is shown a schematic block diagram of an electronics control system 88 for the apparatus according to the invention. System 88 comprises an amplifier stage 92 for detecting and amplifying a signal from electrodes 82 and 84.

A signal power supply unit 94 is provided which generates a plus or minus voltage to be applied across electrodes 82 and 84, for example a voltage of 150 volts is preferred; across the electrodes however this can be somewhat less and can be about 30 volts. Amplifier board 92 provides an earth connection to shield 15 around sample chamber housing 14, and is additionally connected to vacuum transducer 76 and a thermocouple or temperature probe 95 such as a National Semiconductor type LM35CAH device which can be located for measurement of the diluent temperature in second chamber 58.

An amplified sample signal is communicated to a 3½ digit variable potentiometer 96 along line 98 from amplifier stage 92. The potentiometer forms part of an attenuator board 100 which enables adjustment of the signal gain as necessary for the selected orifice size and application. The output from the attenuator board 100 is fed to a 14 bit analogue to digital converter on measurement board 102. The electrode current, vacuum signal and temperature signal are fed along lines 104, 106 and 108 respectively from amplifier board 92 to an 8 bit analogue to digital converter on measurement board 102. Measurement board 102 comprises a channel of 500 kHz or 1 MHz conversions at 14 bits, local storage for a channel of 14 bit data, eight channels of 8 bit (slow) A-D conversion at 100 microsecond conversion, controlling status registers for these devices, 16 bits of digital output control and 16 bits of digital input control.

Lines 110 and 112 are provided between measurement board 102 and amplifier board 92 respectively to enable reversing of the voltage polarity across the electrodes 82 and 84 and also for commencing and stopping an electrode calibration sequence to be described later.

A series of digital input/output ports are provided from measurement board 102. These are connected at lines 114 to 120 a stepper motor driver 43 which is in turn connected to syringe stepper motor 42. Line 114 carries an opto signal from the syringe drive indicating that the syringe is in its home position. Clock, enable and direction signals are relayed to stepper driver 43 via lines 116, 118 and 120 respectively.

The other input/output ports on measurement board 102 communicate with driver board 122. This board, for

example, controls the opening and closing of valves 51, 56, 66, 74 and 80. It also controls the diluent pump 46 and vacuum pumps 62. The diluent sensor 41 and user wand button 30 are also connected to driver board 122.

A power supply unit 90 provides requisite voltage to drive the system 88. Also, a programmable device such as a computer 103 is provided to drive and communicate with boards 100 and 102 (or other elements as necessary). A peripheral device such as a display and/or printer 124 is also provided.

Referring to figure 11 there is shown a circuit diagram for part of the amplifier board 92. A particle signal amplification path comprises four low noise op-amps IC10, IC11, IC12 and IC13 having frequency selective components which allow bandpass filtration of the signal to remove DC and high frequency noise. Amplifier IC11 has a gain adjustment potentiometer that can be set during manufacture and sealed. Amplifier IC13 provides amplifier board 92 with a DC offset adjustment (say -3.2V) using the resistor and potentiometer network R35, R36, VR3 and R37. An electrode (82 or 84) is connected at CN1 and is AC coupled to op-amp IC10 via capacitor C38. A signal generating voltage source 94 of say 150 volts in this example, connects to the signal path at PL 5 and is controlled by reed relay RL1. Resistors R23 and R22 form a high impedance path in comparison to the diluent electrolyte through the orifice making the source 94 appear as a constant current generator.

The amplified signal output at CN2 passes along line 98 to attenuator board 100 shown in figure 10. An orifice current signal can be derived from DC current flowing through the chain VR4, RL1, R23, R22, R21, R47 and orifice 63 via CN1. The electrode voltage source 94 connects at PL5 and a voltage proportional to the orifice current is expressed across VR4 and R47 and amplified by IC14a. This is further conditioned at amplifier IC14b and output to measurement board 102 via line 104 shown in figure 10.

The operation of the system is as follows. The system is formally calibrated for a given electrode voltage, orifice diameter and diluent using inorganic machined particles of known size or volume. After calibration at manufacture, a further calibration verification can be conducted during use of the system as described later. Both first and second chambers 52 and 58 of the sample chamber 14 are washed by alternately opening valve 51 and 56, and valves 80 and 74 respectively, and pumping diluent using pump 46. For example, chambers 52 and 58 can be filled and emptied using the vacuum system including pump 62 and tube 72 and 78, for example a series of three times before again filling chamber 62 and 58 to enable an electrical calibration test. A series of calibration pulses is applied to one of the electrodes 82 or 84 at CN1 using IC9 shown in figure 11 such as a CMOS 555 timer device. The output voltage at CN1 can be controlled for example at 12 volt peak to peak thereby to enable an impedance measurement across the orifice

which signal might for example be in the order of 1 millivolt for a nominal 30 micron orifice. The pulse width can be in the order of 20 to 40 microseconds for frequencies in the order of 15 to 20kHz, or 100 microseconds for 10 kHz. The mean pulse height and number of pulses are monitored as part of the calibration verification, a total of three repeated calibration measurements can be attempted before the sequence is aborted and an error condition signal provided to the user for example at a VDU display or similar output device 124 connected to board 102. The fluid content of the diluent reservoir 38 is then checked for example using a sensor 41 connected to board 122 shown in figure 10.

Syringe 42 is then loaded by directing the three way valve 40 to relay fluid from reservoir 38 into syringe 42 and driving stepper motor 43 a calibrated number of steps in order to load the syringe unit with a known volume of diluent such as 1 millilitre. The user is then requested to place a disposable sterilised tip 28 on the end of nozzle 24 of wand 26 and press the button 30 to confirm. The user then places the disposable tip in the sample fluid and again operates button 30 and syringe 42 is driven down a pre-programmed amount thereby to draw sample fluid into the disposable tip 28.

The user places the sample wand so that nozzle 24 is aligned in groove 20 and tip 28 protrudes into first chamber 52. First chamber 52 is emptied by opening valve 80 and using pump 62. The operator presses button 30 to initiate a dispense sequence wherein syringe 42 is driven with valve 40 directing fluid through pipe 32 such that the sample and diluent are emitted from wand 26 into first chamber 52. If during this operation, detectors 22 determine that nozzle 24 is not in position or has moved away from this position in channel 20, the system waits for the nozzle to be correctly re-positioned. If the sample aspiration, dilution and presentation to chamber 52 is not completed in a predetermined interval, eg 2 minutes, the measurement is aborted and the sample chamber cleaned and the process re-initiated.

It will be appreciated that it is possible by determining the extent of movement of syringe 42 in any direction to quantify, using stepper motor 43, the amount of sample drawn up through tip 28 and the amount of diluent drawn in to syringe 42 to be mixed therewith, and hence the concentration of sample and diluent can be accurately determined.

Second chamber 58 is primed with fresh diluent through tube 54 by opening valve 56 and operating pump 46 to ensure that it is substantially full. The amount of diluent should be sufficient to enable an electrically conductive path between electrodes 82 and 84 across the flow restrictor 60. Preferably an initial priming of second chamber 58 is conducted by passing diluent through tube 54, as previously described, causing turbulence in chamber 58. This acts to reduce the possibility of blockage of orifice 63.

The vacuum reservoir 64 is evacuated of fluid using pumps 62 such that when valve 80 and 74 close a vac-

uum in the order of 130 mm Hg is established in reservoir 64 as read by transducer 76. For example, one of pumps 62 can be turned off or the associated valve 66 closed, on reaching 90% of the predetermined vacuum pressure and using only one of pump 62 to achieve the predetermined level of vacuum.

Valve 74 is opened with valve 56 closed and accordingly some sample and diluent is drawn through the orifice 63 of flow restrictor 61, that is between chambers 52 and 58. The known voltage is applied to the electrode 82 (or 84). After a stabilising delay of say 2 seconds, the current through the orifice, i.e. between electrodes 82 and 84 is checked at line 104 (see figure 10). The current start value or initial value is stored for use in later comparative checks.

Preferably the large DC voltage is removed and a calibrator reference signal applied to electrode 82 (or 84) as previously described.

The known DC voltage, is applied to electrode 82 and the resulting signal amplified between CN1 and CN2 of figure 11 and output from the amplifier board 92 at line 98 is shown in figure 8. An example of the type of pulse observed when a particle passes through orifice 63, is shown in figure 12. The pulse signal S has a height, or voltage, determined by the volume of the particle. The width W of the pulse is determined by the time for the particle to flow through the orifice, i.e. the flow rate and axial length L of the orifice. The signal also has a characteristic background noise N.

In one example of the operation of the system, the programmable device 103 sends a signal to measurement board 102 to initiate 14 bit data conversion at 16384 conversions every two microseconds each. The gain on the attenuator board 100 can then be adjusted as necessary to bring the signal within a suitable range. When the data is captured, it can be sent from measurement board 102 to be stored for example in computer RAM forming part of device 103. The signal such as shown in figure 10 is analysed by, for example, applying a smoothing algorithm to the raw data to remove high frequency noise.

Within a given set or batch of captured data, the system detects a pulse signal S by an increasing leading edge over time. A minimum number of positive going sample values are required to trigger the search for a corresponding negative going edge thereby to detect a pulse signal S. A potential pulse is rejected if a minimum number of negative going samples is not detected after passing through a peak position. The system measures the width of the signal as the point half way along the leading edge to a point on a trailing edge where the voltage becomes less than the starting point on the leading edge. Knowing the expected particle flow rates, it is possible to detect falling fluid flow through the orifice by an increasing pulse width. This can be indicative of a partial blockage of orifice 63. However, if the pulse width is too narrow, this data is rejected as noise rather than a signal. The pulse height of the signal S can be measured

between a net baseline level in the noise N, adjacent signal S and mean or average, peak value of the signal S. As an alternative, the peak height can be determined simply from the value at the first positive going point in a detected pulse signals and taking the difference between this value and that at the pulse maximum.

The pulse area is calculated by integration of signal S and is stored both for a batch and an accumulative histogram for the sample.

Occasionally, two particles pass through orifice 63 at the same time. This can result in a single pulse representative of the volume of both particles, however, sometimes both pulses are resolvable from one another since a first peak is observed followed by a negative going pulse which does not reach the base line voltage before a second positive going pulse due to the second particle occurs. These incidences can be referred to as primary and secondary coincidence respectively. Preferably, the sample concentration of say a million particles per ml is such that these coincidences very rarely occur, however, pulses from secondary coincidence can still be analysed and form part of the data by extrapolating along the negative going edge of the first pulse to determine a pulse width and also by extrapolating the positive going edge of the second pulse also to determine a pulse width. The initial value at the leading edge of the first pulse can be used in the determination of the height of the second pulse.

For each signal detected in a batch the pulse width and pulse height are stored and added to data for that batch. If it is determined that the batch of data is good, checks carried out are described later, the batch data is added to a cumulative pulse height histogram, which is indicative of the particle volume distribution for the sample, and the cumulative pulse width histogram. In this example each batch of data represents the signals over 32 ms of data capture. Also the number of pulses detected is stored as a current batch value and total for all batches for a given sample (i.e. series of measurements). A further store of noise can also be conducted of say any region of noise not containing a pulse signal S for a given a set of captured data. A current value derived from that before and after the batch of data is stored as a mean value of the two measurements. The current value is output at PCRT of the circuit shown in figure 9, or line 104 of amplifier board 92 shown in figure 10. The level of vacuum in reservoir 76 is also measured for each batch. Batch data is added to overall sample data to provide cumulative sample histograms.

Typically, measurements of a sample are made over a twenty to thirty second time period during which the sample is caused to flow through orifice 63, preferably a particle at a time. The flow can be maintained by repeated evacuation of vacuum reservoir 64 using pump 62 so as to retain an under pressure in the reservoir which draws sample from chamber 62 through to chamber 58, i.e. due to the over pressure of atmospheric pressure at inlet 16 to chamber 52. For example, three



successive evacuations of chamber 64 to a preset pressure might be necessary in a thirty second measurement period, depending on how often a deblocking sequence, described later, is conducted.

Under block conditions, the impedance between electrodes 82 and 84 varies significantly and this affects the gain, of what could be considered the orifice 63, or amplifier stage IC10 and so on. Under these conditions, a pulse signal S can approach that of a square wave. This can be monitored by analysing a detected pulse signal S from the time difference between the signal increasing through two distinct and predetermined voltage thresholds above the DC off-set level, and the time for the signal to return back through the two predetermined voltage levels. If the time difference between passing through the lower levels and the upper levels is substantially equal and non-zero then it is indicative of a block having occurred at orifice 63. Additionally, where a square wave signal is followed by a recovery curve which has a characteristic recovery rate determined by the electronic circuitry and which is positive going, in the sense of signal S shown in figure 12, then this signal is indicative of a very large particle in the vicinity of orifice 63. The pulse is designated a rejected pulse.

The background noise N is also monitored. By analysing the frequency domain of the noise, following Fourier transformation, it is possible to detect a partial block since the gain of the amplifier is affected and anomalous frequency amplification can be seen. If a factor of say two difference in amplitude is seen in any frequency component then the data can be rejected. Accordingly, the comparison between background noise and an average background noise can indicate partial or complete blockage of orifice 63.

To check if a given batch of data is acceptable, a first check can be the number pulses observed in the period of the data capture. If the number of pulses vary significantly in comparison to the mean number of pulses per batch, say by greater than or less than 50%, or alternatively one standard deviation from the mean or slightly more, the data can be rejected. Also, comparison of the mean current value at the start and finish of batch data capture with the start current value can be made. If the difference is greater than say  $\pm 20\%$  then the batch data can be rejected. Additionally, the mean current value compared to the mean current value for the previous batch of data can be checked. If the difference is more than say an increase of 10% or a decrease of 5%, then the second batch data can be rejected. Additionally, comparison of the noise with static limits can be made. For example, using the Fourier analysis if a given frequency component varies by a factor of two in comparison with the mean, then this can be taken as defective data. Comparison of the individual or mean pulse width for a batch of data with the cumulative mean pulse width or with static limits can be made. If the discrepancy is greater than say 10% then the batch of data can be rejected assuming that a significant number of

pulses is detected per batch, say at least 30 and preferably 100 pulses per batch. Static limits can be set by a user. Additionally, the number of rejected pulses, or square wave pulses is monitored, data can be rejected when observing either one or more rejected pulses in a given batch. In the event of any of these events occurring which leads to data rejection, a deblock sequence is preferably initiated.

Preferably, the deblock sequence is initiated by turning off the DC voltage on electrode 82 and applying a pulse sequence which can be the calibration pulse sequence. Accordingly, a high frequency vibration, in the frequency range of say 5 to 20 kHz, is set up within the electrolyte. Chamber 58 is also primed by operating pump 46, opening valve 56, whilst valve 74 is also open. The priming sequence is terminated after a short time period of say 100 milliseconds, and the calibration or ultrasonic pulses are also turned off. The large DC voltage is reapplied to electrode 84 and a time delay of say one second is allowed to elapse to stabilize fluid flow. The data measurement sequence is then recommenced as the measurement board is re-initiated, again the attenuator board is adjusted and an initial current value recorded.

Preferably, the data received immediately preceding a deblock sequence is subtracted from cumulative data and rejected as being inconsistent.

The measurement sequence is repeated until the required quantity of fluid has passed through the orifice. This is determined knowing the size of orifice 63, the flow rate from pulse widths and the duration of measurement. Measurement of a sample can also be stopped when the required number of particles within a set range has been measured, or a fatal error occurs and is not rectified such as the orifice current changing and not returning to the value of the start current, the diluent reservoir becoming empty, the data repetition becoming inconsistent, or the base line noise level rising above an acceptable static level.

Upon completion of a measurement sequence, the sample is evacuated from chamber 52 by opening the valve 80. The sample side of the chamber 52, can then be cleaned through a number of rinse cycles by opening valve 51 and pumping at pump 46.

Preferably, an intelligent washing sequence is performed whereby the concentration of sample particles from a previous sample is monitored i.e. from the number of particle signals detected in a known measurement volume. The apparatus can be calibrated to determine how many rinses are required to clean the chambers following a given concentration of sample.

## Claims

1. Apparatus for determining the size of a particle or cell within a fluid, comprising a sample chamber for the fluid, a flow restrictor comprising an orifice,



a pair of electrodes disposed on opposite sides of the orifice and means for measuring a signal representative of the impedance variation between the electrodes thereby to determine the size of a particle within the fluid passing through the orifice, and further comprising means for detecting blockage of the orifice, whether partial or complete, which detecting means measures the rate of occurrence of signal pulses and compares this rate with a predetermined rate.

2. Apparatus according to claim 1 wherein the detecting means comprises means for monitoring the signal, which means detects occurrence of a predetermined variation of the signal indicative of blockage of the orifice.

3. Apparatus according to claim 2 wherein particle passage through the orifice causes a signal pulse which is measured by the measuring means and wherein the monitoring means determines a width of the signal pulse and compares this width with a predetermined value thereby to detect partial or complete blockage of the orifice.

4. Apparatus according to claim 3 wherein the predetermined pulse width value is determined from an average of previously measured pulse widths.

5. Apparatus according to any of claims 2 to 4 wherein the monitoring means monitors the mean base line value of the signal to determine if a significant drift in mean base line value occurs which is indicative of partial or complete blockage of the orifice.

6. Apparatus according to any of claims 2 to 5 wherein the monitoring means compares the height of an individual signal pulse with a known value corresponding to a particle size in the order of or greater than a predetermined size such as the diameter of the orifice.

7. Apparatus according to any of claims 2 to 6 wherein the monitoring means comprises a saturation pulse or square wave detector.

8. Apparatus according to claim 7 wherein the square wave detector compares the time for a pulse signal to pass through a first and second predetermined value and then return back through the predetermined first and second values.

9. Apparatus according to claim 7 or 8 wherein the square wave detector is arranged to detect a characteristic recovery curve.

10. Apparatus according to any of claims 1 to 9

wherein the monitoring means measures the rate of occurrence of signal pulses and compares this rate with a predetermined rate.

10. Apparatus according to any preceding claim wherein the monitoring means compares the number of detected signal pulses in a given time interval with a predetermined value, such as an average of previous measurements.

11. Apparatus according to any preceding claim wherein the monitoring means analyses the background noise of the signal for predetermined variation, such as amplitude variation within a frequency range.

12. Apparatus according to any preceding claim wherein the detecting means comprises an orifice current detector for determining electrical current flow between the electrodes thereby enabling the monitoring means to compare orifice current with predetermined values.

13. Apparatus according to claim 143 wherein the detecting means compares the orifice current value before or after measuring a signal or the mean of the two with an initial value measured before the signal measurement.

14. Apparatus according to claim 12 or 13 wherein the detecting means determines if the difference is greater than a 10% increase or a 5% decrease.

15. Apparatus according to claim 14 wherein the detecting determines if the difference is greater than 20%.

16. Apparatus according to any one of claims 1 to 15 wherein the measuring means comprises a 14 bit detector, or an analogue to digital converter having 14 bit resolution.

17. Apparatus according to any preceding claim comprising means for applying a calibration signal to one of the electrodes and the monitoring means the signal across both electrodes, the monitoring means being adapted to compare the detected calibration signal with a pre-determined signal.

18. Apparatus according to claim 17 wherein the calibration signal comprises a series of pulses.

19. Apparatus according to claim 17 or 18 wherein the calibration signal is repeated a predetermined number of times to attempt to obtain an acceptable result with the predetermined signal, before providing an alarm to the user.

20. Apparatus according to any preceding claim comprising means for deblocking the orifice by removing a particle at least momentarily held within the orifice.

21. Apparatus for determining the size of particles or cells within a fluid, comprising a sample chamber for the fluid, a flow restrictor comprising an orifice, a pair of electrodes disposed on opposite sides of the orifice and means for measuring a signal representing the impedance variation between the electrode thereby to determine the size of a cell within the fluid passing through the orifice, and further comprising means for deblocking the orifice by causing movement of a particle at least momentarily held within the orifice.

22. Apparatus according to claim 20 or 21 wherein the deblocking means operably creates ultrasonic vibrations in the fluid in the region of the orifice.

23. Apparatus according to claim 20, 21, or 22 wherein a series of electrical pulses is applied to one of the pair of electrodes.

24. Apparatus according claim 23, wherein the pulse frequency is greater than 5 kHz preferably 10 kHz, but also in the range of 10 kHz to 15 kHz, and up to about 20 kHz.

25. Apparatus according to claims 22, 23, or 24 wherein the flow restrictor comprises material which exhibits the piezo-electric effect.

26. Apparatus according to any of claims 20 to 25 wherein the deblocking means comprises fluid propulsion means for effecting fluid flow at the flow restrictor.

27. Apparatus according to claim 26 wherein the fluid propulsion means directs fluid substantially in the reverse direction to fluid flow during signal measurements.

28. Apparatus according to claim 26 or 27 further comprising means within the chamber for directing fluid from the fluid propulsion means towards the flow restrictor.

29. Particle sizing apparatus comprising a first and second chamber separated by a flow restrictor comprising an orifice, means for measuring a signal pulse from a variation in electrical impedance at the flow resistor due to the presence of a particle proximal the orifice, and means for detecting a partial or complete blockage of the orifice which detecting means comprises at least two and optionally enabling selection of any two, from at least three, of the

following:-

means for monitoring the rate of signal pulse detection, means for comparing the width or a signal pulse of an average of signal pulses with a predetermined value such as a static limit or a cumulative average of earlier signals;

means for measuring a change in electrical current across the flow restrictor, means for comparing a detected particle size with a predetermined value such as the size of the orifice,

means for determining the presence of a saturation signal, or means for determining anomalies in the background noise.

30. A sample chamber housing for particle sizing apparatus comprising a pair of recesses each adapted to receive an electrode, an electrode in each recess, and a two-part sealant and adhesive for the electrodes.

31. A housing according to claim 30 wherein the adhesive comprises a structural acrylic adhesive.

32. A housing according to claim 30 or 31 wherein the sealant comprises a silicone based sealant such as RTV silicone rubber.

33. A housing according to claim 30, 31 or 32 wherein the recess is defined in a body of a polymer such as acrylic.

34. A flow restrictor for particle sizing apparatus, comprising an orifice allowing flow of particles there through which orifice is tapered.

35. A flow restrictor according to claim 34 wherein the taper is in the order of 5 to 30 % of the mean orifice diameter.

36. A flow restrictor according to claim 34 or 35 or independent thereof, wherein the material defining the orifice is flexible.

37. A sample chamber housing for particle sizing apparatus comprising a first chamber and a second chamber for holding liquids, a liquid communication passage between the first and second chambers adapted to receive a flow restrictor, an inlet for passing a sample into the first chamber, and an outlet from the first chamber enabling automatic removal of the sample therefrom.

38. A housing according to Claim 37 comprising a second inlet into the first chamber for injecting cleaning liquid into the first chamber, wherein the

inlet is preferably disposed to direct liquid at the restrictor in use.

39. A housing according to Claim 37 or 38 further comprising a liquid inlet to the second chamber preferably disposed to direct liquid at the restrictor in use. 5

40. A housing according to Claim 39 wherein the second chamber tapers towards the liquid communication passage between the first and second chambers thereby to guide liquid towards the restrictor in use. 10

41. A housing according to any preceding claim comprising a liquid outlet from the second chamber for automatic removal of liquid therefrom. 15

42. A housing according to any of claims 37 to 41 wherein the first and/or second chamber comprises substantially smooth surfaces to encourage removal of liquid therefrom. 20

43. Particle sizing apparatus comprising a sample chamber housing having a first chamber and a second chamber for holding liquids, a liquid communication passage between the first and second chambers adapted to receive a flow restrictor, a first and second electrode disposed in the first and second chambers respectively, a sample inlet into the first chamber, an outlet from the first chamber, and means for controlling removal of liquid from the first chamber through the outlet. 25 30

44. Apparatus according to claim 43 further comprising a second liquid inlet into the first chamber for diluent or cleaning liquid for example, and means for controlling flow of liquid through the second inlet. 35 40

45. Apparatus according to claims 43 or 44 further comprising a liquid inlet into the second chamber and means for controlling flow of liquid through the inlet, preferably the flowcontrol means enables a jet of liquid to be directed at the flow restrictor in use. 45

46. Apparatus according to any of claims 43,44 or 45, further comprising a liquid outlet from the second chamber and means for controlling removal of fluid through the fluid outlet from the second chamber. 50

47. Apparatus according to any of claims 43 to 46 wherein the fluid flow control means comprises fluid communication to a reservoir of diluent and/or cleaning liquid, and/or pumps such as a peristaltic pump, and/or valves. 55

48. Apparatus according to any of claims 43 to 47 wherein the fluid removal control means comprises a pump system such as a vacuum pump.

49. Apparatus according to any of claims 43 to 48 comprising means for effecting a pressure differential between the first and second chambers preferably comprising means for controlling pump and/or a valve and/or a pressure transducer for monitoring the pressure differential.

50. Particle sizing apparatus according to any of claims 43 to 49 comprising a wand, means for drawing liquid into the wand and means for expelling liquid from the wand.

51. Apparatus according to claim 50 wherein the means for drawing and expelling liquid comprises a pump and valve arrangement.

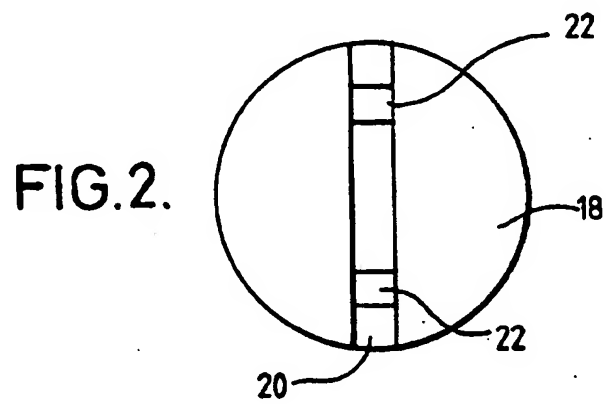
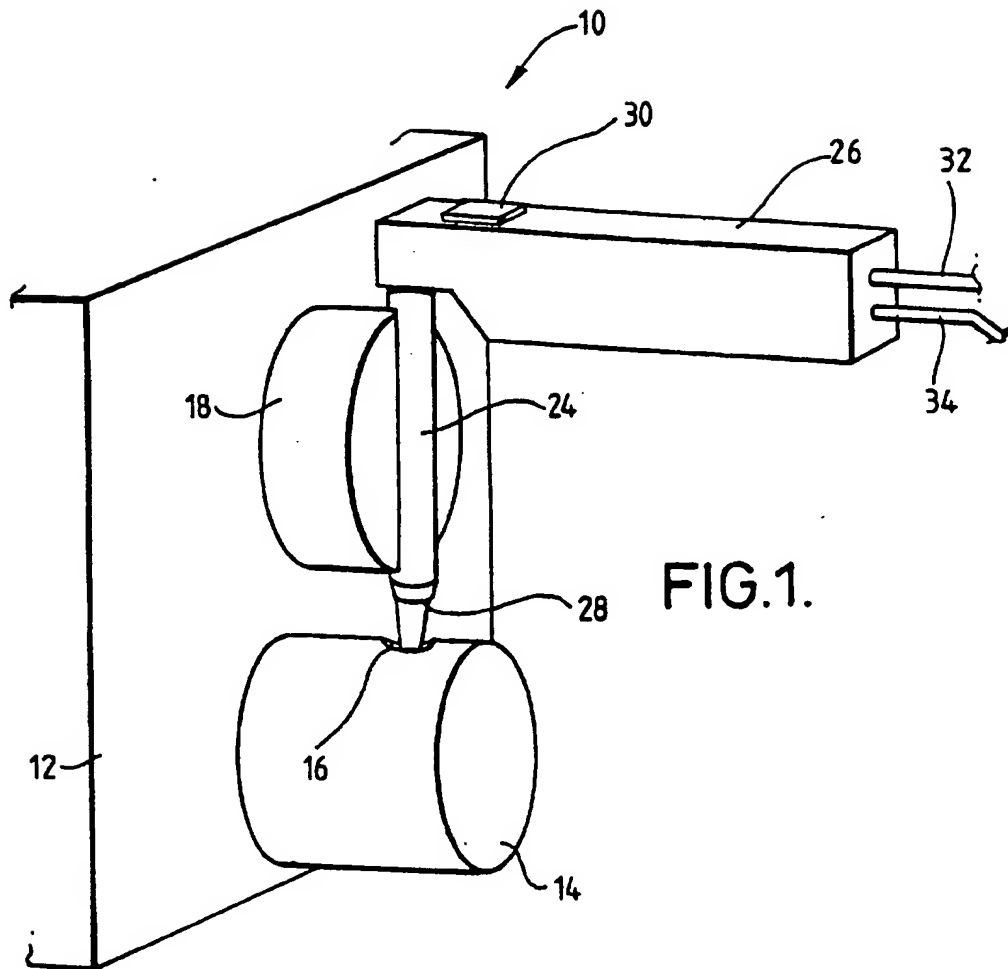
52. Apparatus according to claim 51 wherein the pump comprises a controlled metering device such as a syringe driven by a steppemotor.

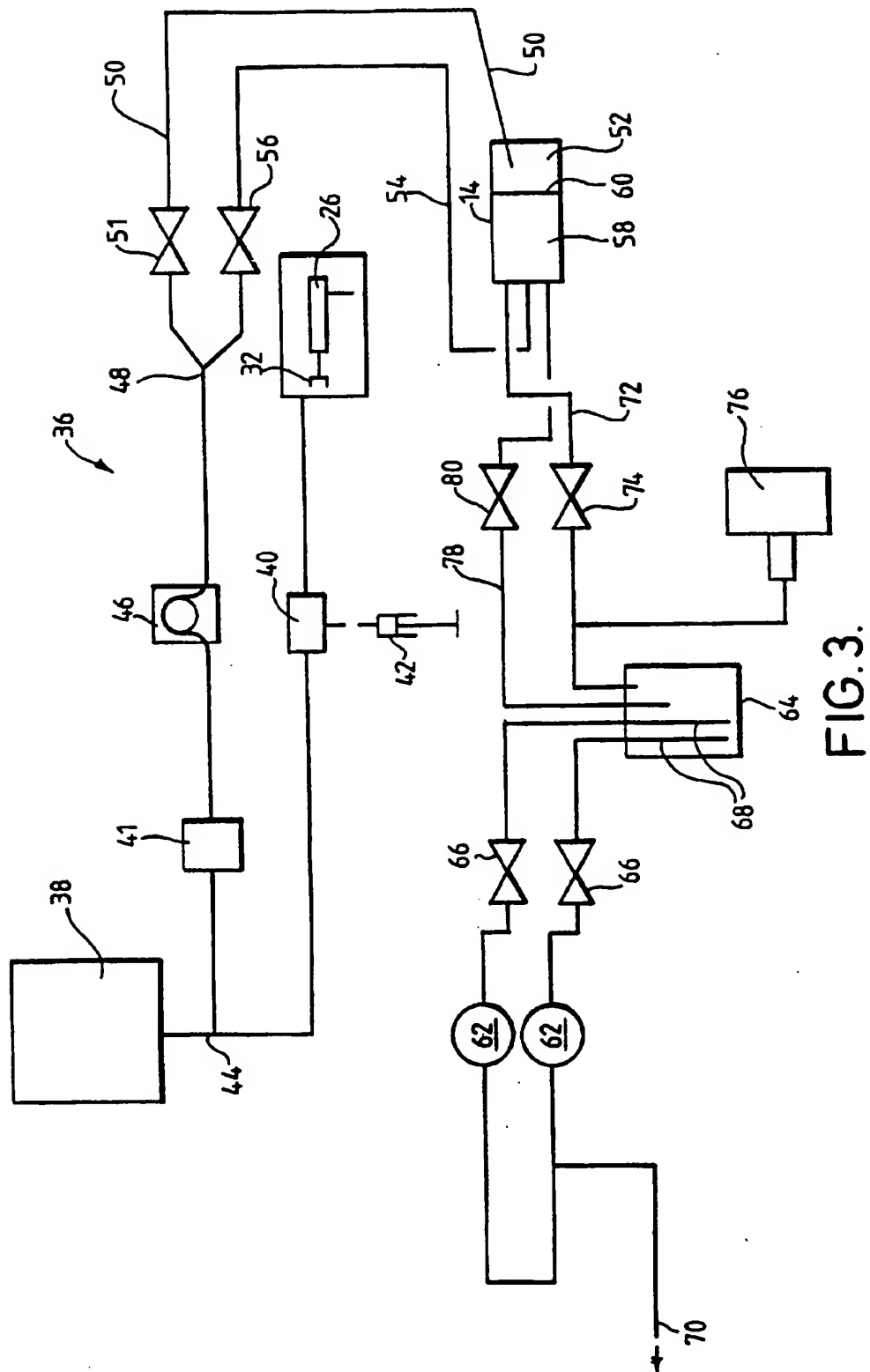
53. Apparatus according to claim 51 or 52 wherein the valve arrangement comprises a three-way valve enabling fluid to be drawn or expelled along at least two outlets from the valve.

54. Apparatus according to any of claims 52 or 53 further comprising means for ensuring that the wand is correctly positioned for inserting a sample into a sample chamber.

55. Apparatus according to any of claims 1 to 20 wherein the signal pulse rate is determined by means for capturing one or more signal pulses over a period of time, or in a memory block, and determining the number of signals detected in the period of time, preferably means is provided for comparing the detected number with a finite value or an earlier result, in order to determine if the orifice is blocked.

56. Apparatus according to any of claims 20 to 29 wherein the deblocking means is actuated periodically, for example after a predetermined time, after a number of signal measurements, or where periodic signal measurements are temporarily stored in a transient memory, after each period of measurements, the apparatus thus comprising means for periodically actuating the deblocking means.





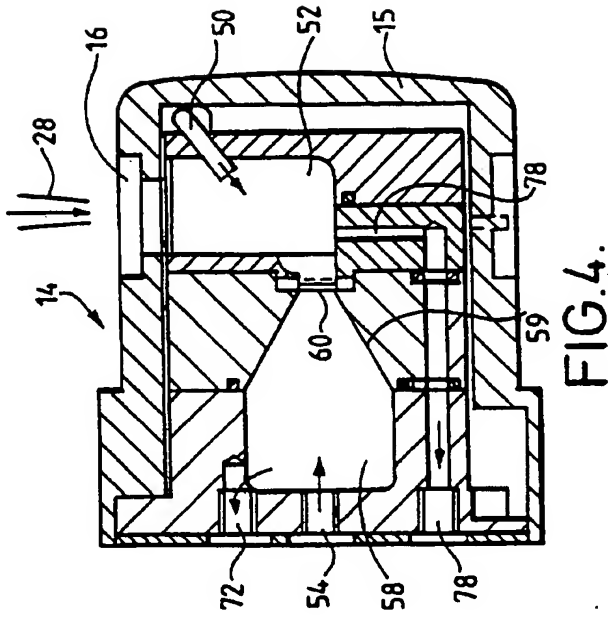


FIG. 4.

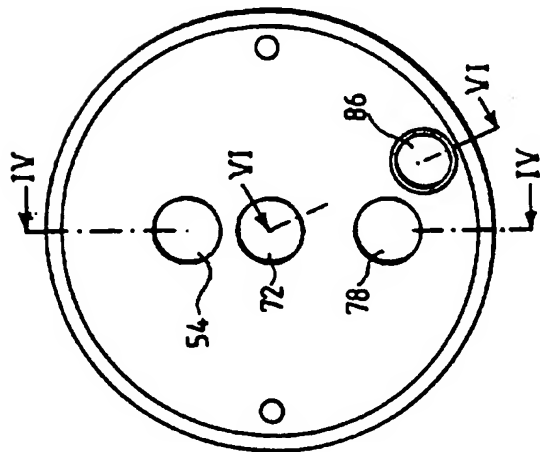


FIG. 5.

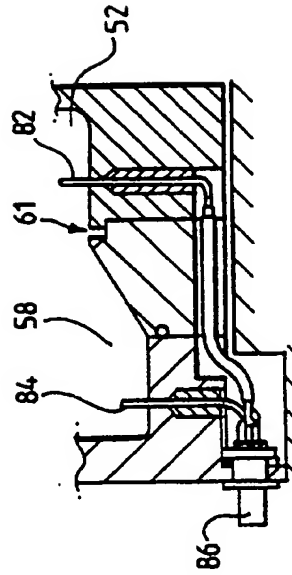
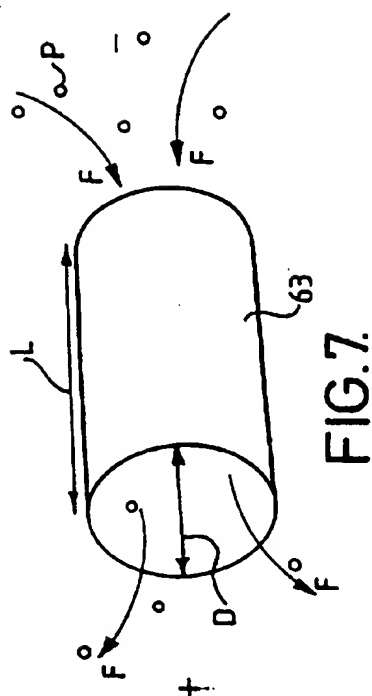
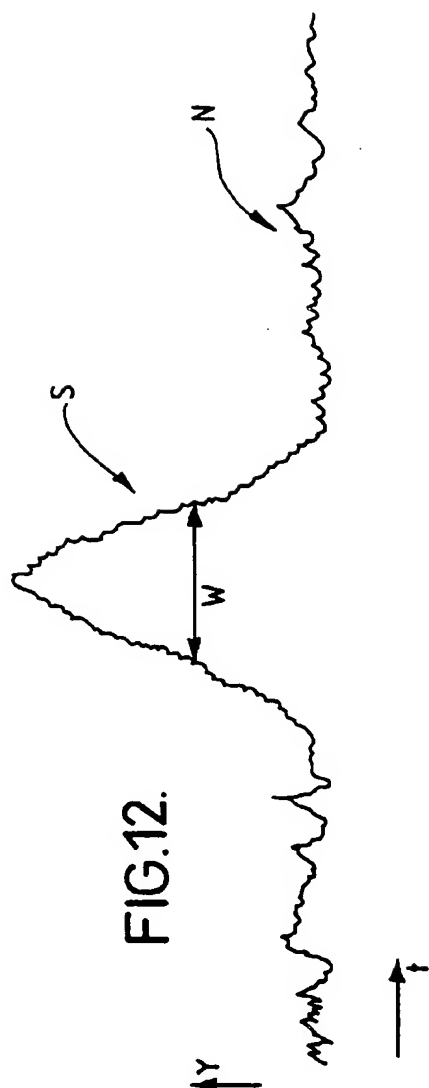


FIG. 6.





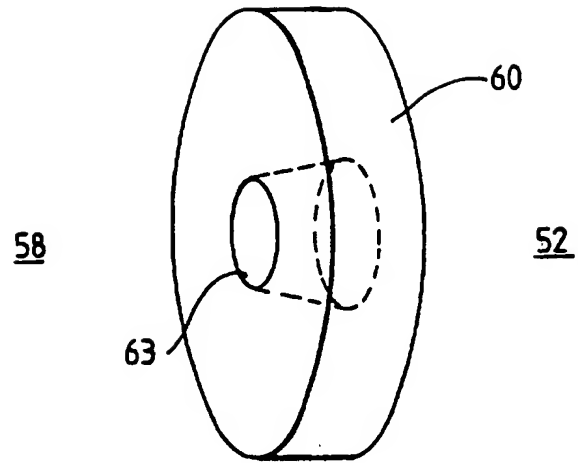


FIG. 8.

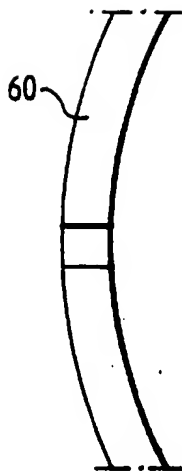


FIG. 9(a)

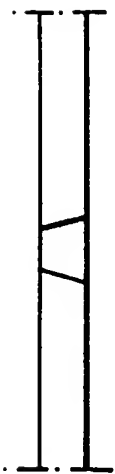
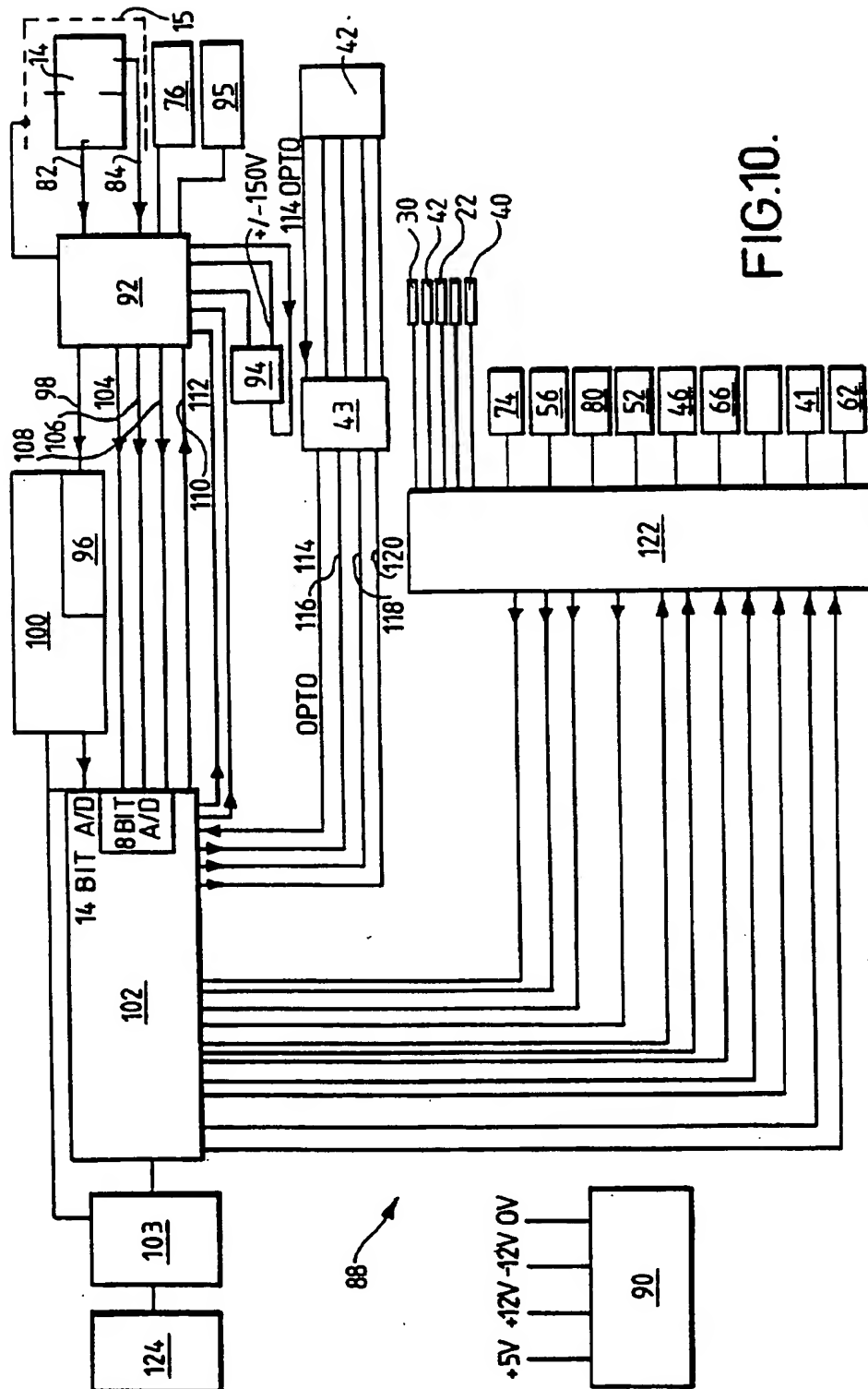


FIG. 9(b)



FIG. 9(c)



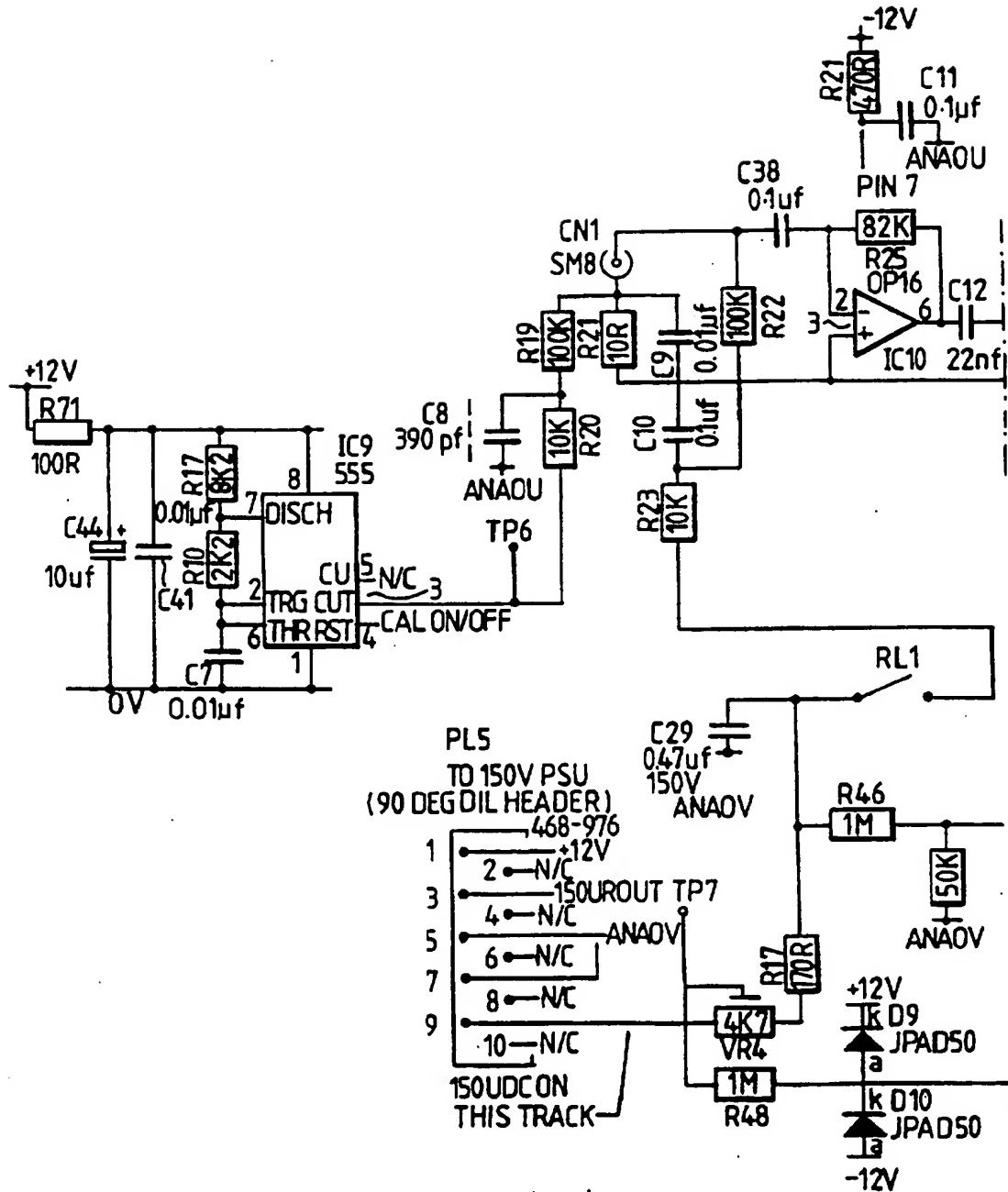


FIG.11a.

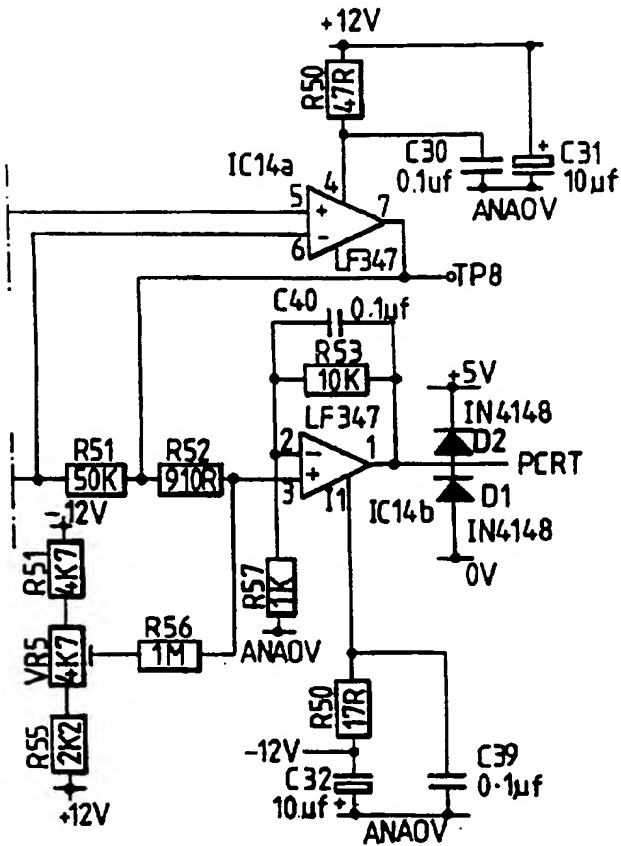
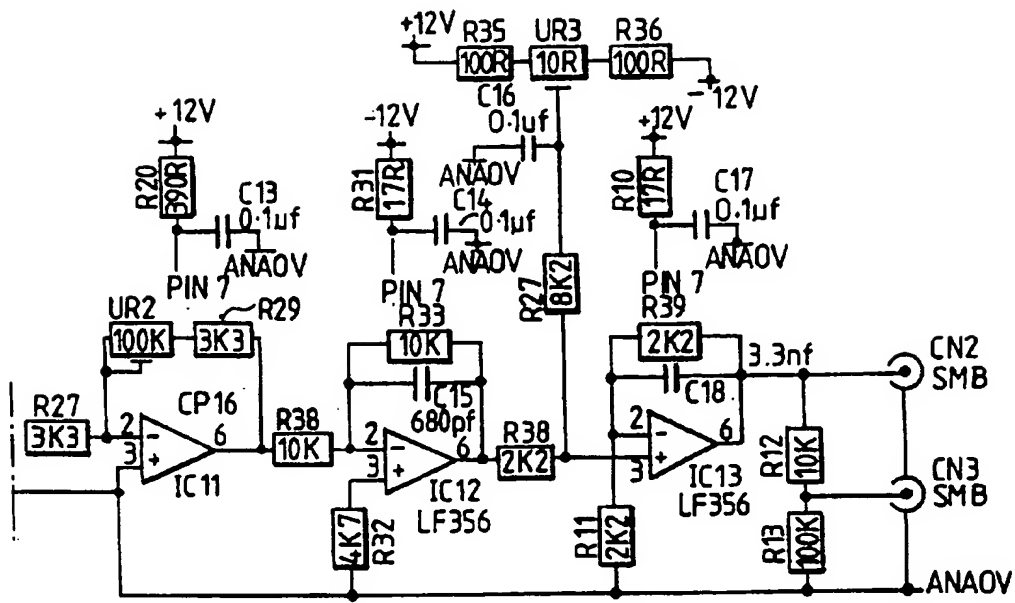


FIG.11b.